Transcutaneous electrical stimulation applied to the infratrochlear nerve induces a homatropine-resistant miosis in humans

B. M. FUSCO, M. ALESSANDRI, V. CAMPAGNOLO AND M. FANCIULLacci*
Institute of Internal Medicine and Therapeutics IV, and *Department of Preclinical and Clinical Pharmacology, University of Florence, Florence, Italy

(Received 25 July/8 December 1989; accepted 5 January 1990)

SUMMARY

1. Both high- and low-intensity transcutaneous electrical stimuli were applied to the emergence of the infratrochlear nerve in 18 healthy subjects. The effect on the size of the homolateral pupil was investigated. The width of the pupil was also measured when high-intensity transcutaneous electrical stimulation was applied to the contralateral side.

2. The high-intensity pulse resulted in constriction of the pupil when the stimulation was homolateral. The miosis was slow in onset (120 s latency) and long-lasting (80 s). No pupillary changes were detected after either ipsilateral low-intensity or contralateral high-intensity stimuli.

3. In 11 healthy subjects, the pupillary response to transcutaneous electrical stimulation was evaluated during iris parasympathetic blockade induced by homatropine eyedrops. The disappearance of the light reflex due to homatropine was considered an index of the parasympathetic blockade. Afterwards, a high-intensity pulse was transcutaneously delivered to the emergence of the infratrochlear nerve and the ipsilateral pupil size was measured.

4. A reduction in the pupillary size followed the electrical stimulation, still under the effect of homatropine which abolished the light reflex. The time course of this pupillary constriction was similar to that seen without the influence of homatropine.

5. The findings suggest that homolateral miosis, observed after unilateral high-intensity stimulation of the infratrochlear nerve, does not stem from cholinergic activation. It has been suggested that miosis induced by transcutaneous electrical stimulation may be due to an antidromic activation of the iris sensory fibres.

Key words: axon reflex, homatropine, iris sensory neurons, light reflex, miosis, pupil, transcutaneous electrical stimulation.

Abbreviations: SP, substance P; TES, transcutaneous electrical stimulation.

INTRODUCTION

The size of the pupil is determined by a balance of the innervation between the autonomically supplied sphincter and the radially arranged dilator muscles of the iris; the sphincter muscle plays the major role. The commonest stimulus for pupillary constriction, which involves cholinergic pathways, is the exposure of the retina to light. Moreover, reflex pupillary constriction allows for the act of convergence and accommodation to nearby objects. As a rule, noxious stimuli induce a mydriatic response [1]. However, such stimuli, when directed to the ocular area, can result in a pupillary constriction. In particular, the so-called 'oculo-trigeminal' reflex, elicited by sustained stimulation of the cornea and conjunctiva or by pinching or pricking the skin of the eyelid, consists of a brief bilateral dilatation followed by a prolonged constriction of the pupil. The miotic response is noticeable in both eyes, even though the pupil of the stimulated side is usually narrowed to a greater extent. The structures which are involved in this response are unknown. However, it has been suggested that impulses run through the trigeminal afferents and the trigeminal sensory nucleus; thus, in some unknown way, pupillary contraction is mediated by the activation of a cholinergic mechanism [2].

Hypotheses have been advanced that the trigeminal sensory neurons arising from the iris may themselves be involved in the mechanism of iris constriction through an antidromic activity, especially after ocular injury. In 1910, Bruce [3] extended to the iris the concept inferred by the observation of the antidromic sensory vasodilatation [4] and explained the ocular response to local injury, including miosis, by an axon reflex. The role of the capillary
dilatation, induced by an axon reflex, was emphasized in 1943 by Longworthy & Ortga [5] to explain the changes in pupillary size during ocular irritation. In 1957, Perkins [6] found that electrical stimulation of the ophthalmic trigeminal branch of the rabbit resulted in a miosis similar to that provoked by local injury. He hypothesized that an unidentified substance, capable of contracting the pupil and which he called 'iradin', might be released after an antidromic activation of the iris trigeminal neurons. In 1979, Bill et al. [7] showed that miosis after electrical stimulation of the trigeminal branch is related to an increase in substance P (SP), one of the putative neurotransmitters of sensory neurons [8], in the anterior chamber of the eye. Jampol et al. [9], Butler & Hammond [10] and Butler et al. [11] stated that sensory denervation prevents miosis caused by eye exposure to both chemical and physical noxious stimuli. They have concluded that sensory fibres mediate the pupillary response. Holmdahl et al. [12] showed that the iris constriction occurring during the ocular inflammatory response is inhibited by an antagonist of SP. Moreover, SP and neurokinin A, which are found in certain sensory neurons of various mammalian species [13-15], have a potent miotic effect on the rabbit eye [16]. In a recent preliminary study, we showed that in man too, locally applied SP induces pupillary constriction [17].

The present study was designed to evaluate, in healthy volunteers, the pupillary response to transcutaneous electrical stimulation (TES) of an area included in the ophthalmic trigeminal distribution. TES was applied alternatively to both homolateral and contralateral sides of the pupil being examined. The emergence of the infra-trigeminal nerve was chosen as the stimulation site, because of the anatomical closeness of the infra-trigeminal nerve fibres to the sensory fibres which arise from the iris. In fact, both bundles of fibres run within the nasociliary trunk of the trigeminal ophthalmic branch. Moreover, the response to both low- and high-intensity pulses was assayed to verify whether the pupillary variations are related to the intensity of the electrical stimulus. Finally, the cholinergic involvement in the pupillary response to electrical stimulus was studied by administering high-intensity TES after conjunctival instillation of homatropine in the eye being examined.

METHODS
The study was carried out on 29 healthy subjects, who were either medical students or members of the medical staff of our Institute. Informed consent was obtained from each subject before the start of the study, which had been fully approved by the Supervisory Committee of our Institute. Before admission to the study, all subjects were screened in order to exclude any eye disease. A single eye from each volunteer was chosen at random for the investigation.

Pupillary response to TES
This part of the study involved 18 subjects (10 men and eight women, mean age ± SEM 37.4 ± 4.1 years). The effects of low- (10 mA) and high- (40 mA) intensity TES on the area of the pupil homolateral to the side of the stimulation were evaluated. Moreover, the response to high-intensity TES, administered to the opposite side of the pupil being measured, was also assayed. Each test was performed on 3 different days, always starting at 09.00 hours. Pupil area was measured by using a TV monocular electronic pupillometer (Hamamatsu, Japan) consisting of a TV camera, a chin and forehead rest table, a control unit and a monitor. An eye-fix lamp, producing a standard green light, was included in the TV camera.

Measurements were carried out in infrared light conditions and were started after a 5 min period of adaptation to the darkness. First, subjects were requested to focus on the green light, which was considered the standard reference point for accommodation. Then the measurement of the pupil area was performed. After the basal measurement (pre-stimulation value), an electrical stimulus, consisting of a single square wave pulse of 0.8 ms duration, was applied to the cutaneous area corresponding to the emergence of the infratrochlear nerve. The pulse was administered by means of a monopolar electrolyte-gel sponge electrode connected to a pulse generator (Neuroton Siemens, Erlangen, West Germany). During the first two study sessions, the area of the pupil was monitored every 20 s for 4 min after the administration of electrical pulse on the same side. In one of these two sessions, chosen at random, subjects received a high-intensity stimulus, whereas during the other session a low-intensity pulse was given. In a third session, the high-intensity stimulus was applied to the side contralateral to the pupil being examined and the response was evaluated for 4 min at the time intervals mentioned above.

Pupillary response during homatropine-induced blockade of the light reflex
This study was performed on 11 volunteers (six men and five women, mean age ± SEM 40.3 ± 5.1 years). In these subjects the effects of high-intensity TES applied to the emergence of the infra-trigeminal nerve were assayed in the pupil of the ipsilateral eye, which had been previously treated (90 min before) with homatropine hydrobromide eyedrops (1% aqueous solution). The disappearance of the light reflex was assumed to be an indication of homatropine-induced blockade of parasympathetic activity. To evaluate this block, before and during the homatropine effect, a well-tolerated brief light, stimulus (time 0.5 s, intensity 100 ± 10 nW) was administered while the pupil area was measured. The light-induced miosis was directly detected by the pupillometer which displayed the values of the pupil areas measured before (A₁) and after (A₂) the light stimulation and then the percentage difference between the two areas (A₃) was calculated. Immediately after the evaluation of the homatropine-induced block of the reflex to light, the response of the pupil to homolateral high-intensity TES was evaluated by the above-described procedure. A control session was performed 1 week later, during which homatropine was re-administered in the same eye of each subject, and the measurement of the
Miosis induced by electrical stimulation in humans

Pupil area was repeated by following the entire test procedure, without, however, administering any electrical stimulation.

Statistical analysis

Analysis of variance for repeated measures was used to test differences in average pupil areas detected during the first part of the study; the factors were: the stimuli (homolateral low- and high-intensity and contralateral high-intensity pulses), the time, and the interaction between them (type of stimulus x time). Afterwards, point and level of significance were analysed by applying post-hoc tests. Comparison between the values measured before and after each stimulation was made by using Dunnett’s test. Duncan’s test allowed point by point comparison of the values of the pupil area which were observed during the three kinds of electrical stimulation.

In the second part of the study, the homatropine-induced block of pupillary response to the light stimulus was analysed by comparing the values of \( A_1 \) and \( A_2 \) detected before eyelid instillation with those obtained after drug administration. For this purpose, the \( t \)-test for paired data was used. The \( t \)-test for paired data was also employed to compare the pupil area values which were observed during the three kinds of electrical stimulation.

Values of \( P \) smaller than 0.05 were set as the significance level for hypothesis testing.

RESULTS

Pupillary response to TES

The pupil areas measured after both homolateral application of low-intensity TES and contralateral administration of high-intensity TES did not show any statistical difference when compared with the respective baseline values (Table 1). The homolateral high-intensity stimulus induced a reduction in pupil width which was statistically significant at 120, 140, 160, 180 and 200 s (\( P<0.01 \)) when compared with pre-stimulation values (Table 1).

By comparing the three groups of data, the baseline values appeared statistically matched. A significant reduction in the pupil area measured after the homolateral high-intensity electrical stimulation was observed when compared with those detected after homolateral low-intensity and contralateral high-intensity TES. The reduction in the pupil size was statistically significant 100 (\( P<0.05 \)), 120, 140, 160, 180 (\( P<0.01 \)), and 200 (\( P<0.05 \)) s after the stimulation (Fig. 1).

Pupillary response to TES under the effect of homatropine

Ninety minutes after application of homatropine eye-drops, a marked mydriasis and disappearance of the light reflex was obtained in the treated eye (Table 2). Under the effect of homatropine, the values of the pupil area detected before the high-intensity TES were not statistically different from the basal values detected during the
Fig. 1. Pupil areas measured before and after homolateral low- (○) and high- (▲) intensity TES and contralateral high-intensity TES (●) applied transcutaneously to the emergence of the infratrochlear nerve. Analysis of variance for repeated measures revealed significant effects of the high-intensity stimulus (F= 6.75, P< 0.01) and time (F= 10.72, P< 0.001), as well as a significant stimulus x time interaction (F= 13.82, P< 0.001). Statistical significance (Duncan’s test): * P< 0.05; ** P< 0.01 vs both homolateral low-intensity and contralateral high-intensity TES. All data are means ± SEM (n= 18).

DISCUSSION

TES, applied to the cutaneous emergence of the infratrochlear nerve, resulted in a long-lasting and sustained miotic response which occurred only in the ipsilateral pupil. Pupillary constriction was achieved by administering a single high-intensity pulse, whereas low-intensity stimulus was incapable of evoking pupillary change. Since a recruitment of the thinner afferent neurons is obtained by increasing the intensity of electrical stimulation [18, 19], our findings suggest that an activation of the smaller sensory neurons is needed to elicit the pupillary effect.

The occurrence of miosis exclusively in the stimulated side indicates that the response does not necessarily involve the central nervous structures. In fact, an activation of the brainstem nucleus should be followed by a mutual narrowing of the contralateral pupil. Moreover, pupillary constriction, with a similar time course, occurred even after the conjunctival instillation of a dose of homatropine capable of disabling the cholinergic reflex to light; therefore, the miosis induced by the high-intensity stimulus does not seem to depend on a parasympathetic mechanism.

The activation of larger diameter afferent fibres produces an inhibition of the sympathetic neurons [20]; thus miosis after electrical stimulation might stem from a reduction in the iris sympathetic tonus through the activation of these afferents. However, it seems unlikely, since no pupillary variation was observed after applying low-intensity stimulation which is capable of exciting the larger afferent neurons.

The hypothesis that trigeminal fibres contribute to control of pupil size suggests an interesting interpretation of the present findings. The time course of the pupillary response to high-intensity TES is slow in onset and long-lasting: it is not comparable with that occurring in autonomic-mediated reflexes. On the contrary, it correlates with the response found in the isolated rabbit iris after transmural electrical stimulation, which results in a non-cholinergic, non-adrenergic constriction that is inhibited by SP antagonists [21]. Moreover, it is also similar in onset and duration to the pupillary constriction induced in the rabbit by direct stimulation of the ophthalmic nerve [7]. Most of the evidence on the dual ‘sensory-effector’ function of sensory fibres, in the iris particularly, stems from experimental studies in animals. In man, SP-like immunoreactivity is found in ocular structures [22], including the iris sphincter muscle. The peptide, instilled in the conjunctival sac of healthy volunteers, produces consistent miosis [17].

The proposal that miosis induced by TES is mediated by an anterograde discharge of iris sensory fibres leads to speculation as to the mechanisms through which antidromic activation may be elicited. It has been suggested that TES may antidromically activate the sensory fibres. The increased survival of the ischaemia muscle-cutaneous flap in the rat, observed after local application of high-intensity, high-frequency TES, provides evidence for an antidromic activation of the sensory fibres [23]. In fact, the effect is abolished by pharmacological sensory denervation and reproduced by local application of calcitonin-gene-related peptide [24], a vasoactive polypeptide [25] which is contained in the sensory fibres and released after antidromic stimulation [26]. Therefore, miosis induced by TES may be due to a preganglionic short loop, acting through an axo-axonal reflex [27], between two groups of sensory fibres, the fibres arising from the area innervated by the infraorbital nerve and those coming from the iris. In fact, both bundles of fibres join in the same trunk (nasociliary) of the ophthalmic branch. Otherwise an intraganglionic integration through an inter-

**Table 2. Blockade of the light reflex during the homatropine-induced miosis**

<table>
<thead>
<tr>
<th></th>
<th>Before homatropine</th>
<th>After homatropine</th>
</tr>
</thead>
<tbody>
<tr>
<td>A₁ (mm²)</td>
<td>20.64 ± 1.71</td>
<td>35.6 ± 1.2*</td>
</tr>
<tr>
<td>A₂ (mm²)</td>
<td>11.2 ± 1.23</td>
<td>34.7 ± 1.17*</td>
</tr>
<tr>
<td>A₃ (%)</td>
<td>45.74 ± 5.52</td>
<td>2.58 ± 0.30*</td>
</tr>
</tbody>
</table>

The pupil area was measured before (A₁) and after (A₂) light stimulus. A₁ represents the percentage difference between A₁ and A₂. Values are means ± SEM (n= 11). Statistical significance (paired t-test): * P< 0.001 compared with before homatropine.
somatic connection, acting like an axon reflex [28], may occur. Finally, a spreading of the electrical pulse directly from the stimulated cutaneous area to the iris sensory fibres must be taken into account as a possible mechanism of the antidromic activation.

In conclusion, the application of a single high-intensity electrical stimulus on the cutaneous emergence of the infratrochlear nerve elicits a non-cholinergic miotic response only in the pupil of the stimulated side. The onset, duration and unilaterality of the pupillary constriction do not relate this phenomenon to the commonly known pupillary reflexes. Though the interpretation is uncertain, miosis induced by TES appears to be a potential tool with which to assay the function of the iris innervation. The results obtained by using this testing method on patients affected from diseases, such as cluster headache, which presumably involve iris sensory neurons, seem to support its clinical usefulness [29].

ACKNOWLEDGMENTS

This study was supported by the Research National Council (Rome, Italy), Medicina Preventiva e Riabilitativa, sottoprogetto ‘Controllo del Dolore’ grant no. 87.00272.56, and by the Ministry of Education of Italy. We thank Ms Sherry Sachenou for revision of the English.

REFERENCES

5. Longworthly, O. & Ortaga, L. Innervation of the iris of the albino rabbit as related to its function; theoretical discussion of abnormalities of the pupils observed in man. Medicine 1943; 22, 287–98.


